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Inonotus griseus sp. nov. from eastern China

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Abstract —*Inonotus griseus* is described and illustrated from Anhui Province, eastern China. This new species is distinguished by its annual and resupinate basidiocarps with a grey cracked pore surface, a monomitic hyphal system in both subiculum and trama, the presence of subulate to ventricose hymenial setae, the presence of hyphoid setae in both subiculum and trama, and ellipsoid, hyaline, slightly thick-walled cyanophilous basidiospores (9–10.5 × 6.3–7.2 µm). ITS sequence analyses support *I. griseus* as a distinct lineage within the core clade of *Inonotus*.

Key words — *Hymenochaetaceae, Hymenochaetales*, *Basidiomycota,* polypore, taxonomy

Introduction

Inonotus P. Karst. (*Hymenochaetaceae*) was typified by *I. hispidus* (Bull.) P. Karst. Although more than 100 species have been accepted in this genus in a wide sense (Ryvarden 2005), molecular phylogenies have supported some *Inonotus* species in other small genera, including *Inocutis* Fiasson & Niemelä, *Inonotopsis* Parmasto, *Mensularia* Lázaro Ibiza, and *Onnia* P. Karst. (Wagner & Fischer 2002). Dai (2010), accepting this taxonomic segregation, morphologically emended the concept of *Inonotus*. Current characters of *Inonotus* sensu stricto include annual to perennial, resupinate, effused-reflexed or pileate, and yellowish to brown basidiocarps, homogeneous context, a monomitic hyphal system (at least in context/subiculum) with simple septate generative hyphae, presence or absence of hymenial and hyphoid setae, and ellipsoid, hyaline to yellowish or brownish, thick-walled and smooth basidiospores (Dai 2010).

More than 1200 wood-decaying basidiomycetes have been reported from China (Dai 2011, 2012a). The taxonomy of *Hymenochaetaceae* has been extensively studied in China (Zhou & Jia 2010, Zhou & Dai 2012, Zhou & Xue 2012, Cui & Decock 2013, Qin & Zhou 2013, Zhou 2013, 2014a,b,c,d, Zhou &

Qin, 2013, Zhou et al. 2014a), and 38 species of *Inonotus* have been reported here (Dai 2010, 2012b, Cui et al. 2011, Dai et al. 2011, Wu et al. 2012, Zhou & Qin 2012, Tian et al. 2013, Yu et al. 2013). However, there are still many Chinese specimens within *Hymenochaetaceae* that have not been identified to species level. Of these specimens, one is described and illustrated here as a new species of *Inonotus*.

Materials & methods

Morphological examination

Our specimen is deposited in the herbarium of the Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, China (IFP). The microscopic procedure follows Zhou et al. (2014b). Specimen sections were prepared with cotton blue (CB), 5% potassium hydroxide (KOH), and Melzer's reagent (IKI), and then examined using a Nikon Eclipse 80i microscope under magnifications up to 1000× and phase contrast illumination. Measurements and drawings were made from sections stained with CB. When describing the basidiospore size variation, the upper and lower 5% of measurements were excluded from the range, with extreme values enclosed in parentheses. Line drawings were made with the aid of a light tube. Special color terms follow Petersen (1996). The following abbreviations are used: IKI– = neither amyloid nor dextrinoid; CB+ = cyanophilous; L = mean basidiospore length (arithmetic average of all basidiospores); W = mean basidiospore width (arithmetic average of all basidiospores); Q = the ratio of L/W; and n = number of basidiospores measured from given number of specimens.

Molecular sequencing

The ITS sequence of our specimen was directly amplified using Phire® Plant Direct PCR Kit (Finnzymes Oy, Finland) in accordance with the manufacturer's instructions with primers ITS5 and ITS4 (White et al. 1990). The PCR procedure was as follows: initial denaturation at 98°C for 5 min, followed by 39 cycles at 98°C for 5 s, 59°C for 5 s and 72°C for 5 s, and a final extension of 72°C for 10 min. The amplicon was sequenced in Beijing Genomics Institute, China with the same primers as in amplifications. The newly generated sequence was deposited at GenBank (http://www.ncbi.nlm.nih.gov).

Phylogenetic analysis

To explore the phylogenetic position of our specimen, 35 additional ITS sequences of *Inonotus* (Fig. 1) were retrieved from GenBank. The ITS sequence of *Phellinus populicola* Niemelä was selected as outgroup (Wagner & Fischer 2002). The dataset was aligned using MAFFT 7 (Katoh & Standley 2013) with Q-INS-i option (Katoh & Toh 2008). The resulting alignment was subjected to phylogenetic analysis using maximum likelihood (ML) and maximum parsimony (MP) algorithms. raxmlGUI 1.2 (Stamatakis 2006, Silvestro & Michalak 2012) with GTR + I + G model and auto FC option (Pattengale et al. 2010) in bootstrap (BS) replicates was used to construct ML trees. MP analysis was conducted using PAUP* 4.0b10 (Swofford 2002) with heuristic search. All characters were equally weighted and gaps were treated as missing data. Other parameters were set as follows: starting tree obtained via stepwise addition, tree-bisection-reconnection

branch swapping, steepest descent option not in effect, and 'MulTrees' option in effect. The resulting trees were tested by 1000 BS replicates. As the topologies from ML and MP analyses were congruent, only the topology from the ML analysis is presented along with BS values simultaneously ≥50% from both analyses.

Results

An alignment of 957 characters resulted from the ITS dataset of 37 sequences. Of these characters, 524 are constant, 116 parsimony-uninformative, and 317 parsimony-informative. A total of 250 BS replicates was performed to test the ML tree, and two equally most parsimonious trees of 1217 steps (CI = 0.569, RI = 0.757) were retained. The ITS-based phylogenetic tree (Fig. 1) recovered at least three strongly supported *Inonotus* clades, and the sequence from our specimen was nested as a distinct lineage within the core clade that included the generic type, *Inonotus hispidus*.

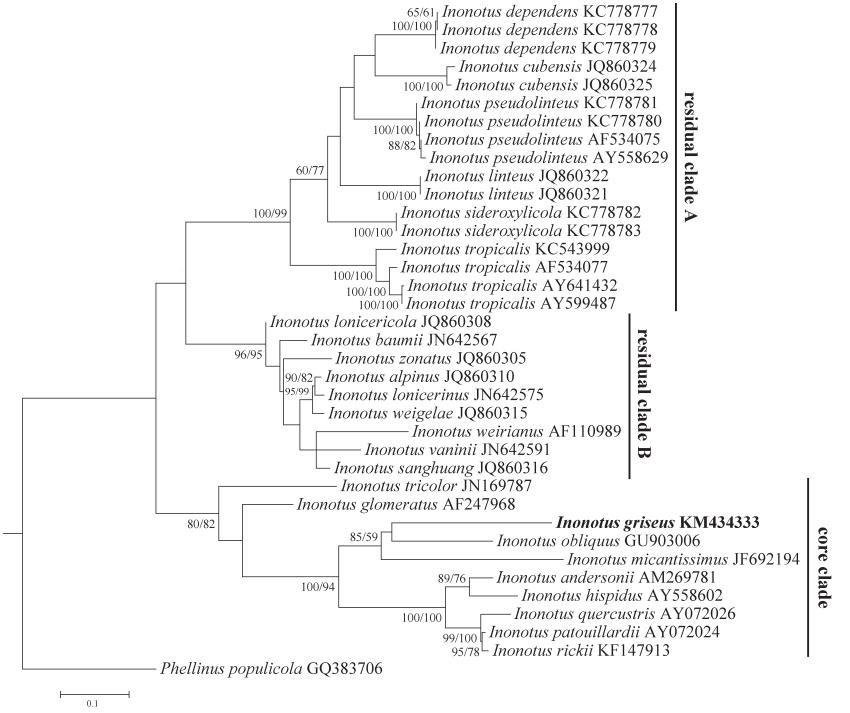

Figure 1. Phylogenetic position of *Inonotus griseus* inferred from ITS sequences. Topology is from maximum likelihood analysis, and the bootstrap values simultaneously ≥50% from maximum likelihood/maximum parsimony analyses are indicated at the nodes.

Figure 2. *Inonotus griseus* (holotype) in situ.

Taxonomy

Inonotus griseus L.W. Zhou, **sp. nov.** Figs. 2, 3

MycoBank MB 810088

Differs from *Inonotus chihshanyenus* by its greyish brown to dark grey pore surface, smaller pores, shorter hymenial setae, and larger basidiospores.

Type: China. Anhui Province: Huangshan, Qimen County, Guniujiang Park, on dead angiosperm tree, 10.VIII.2013, L.W. Zhou LWZ 20130810-20 (**holotype**, IFP; GenBank KM434333).

Etymology: *griseus* (Lat.): refers to the grey pore surface.

Basidiocarps annual, resupinate, adnate, inseparable, without odor or taste when fresh, woody, up to 20 cm long, 9 cm wide, and 1.5 cm thick. Pore surface greyish brown to dark grey, sometimes black at the margin, slightly glancing, cracked; sterile margin narrow, up to 0.5 mm, cinnamon-buff; pores round to angular, 4–6 per mm or even 1–2 per mm at the margin; dissepiments thick, entire. Subiculum yellowish brown, soft, very thin to almost lacking, up to 0.5 mm. Tubes honey-yellow to cinnamon-buff, woody, up to 1.5 cm long.

Hyphal system monomitic; generative hyphae simple septate; tissue darkening but otherwise unchanged in KOH.

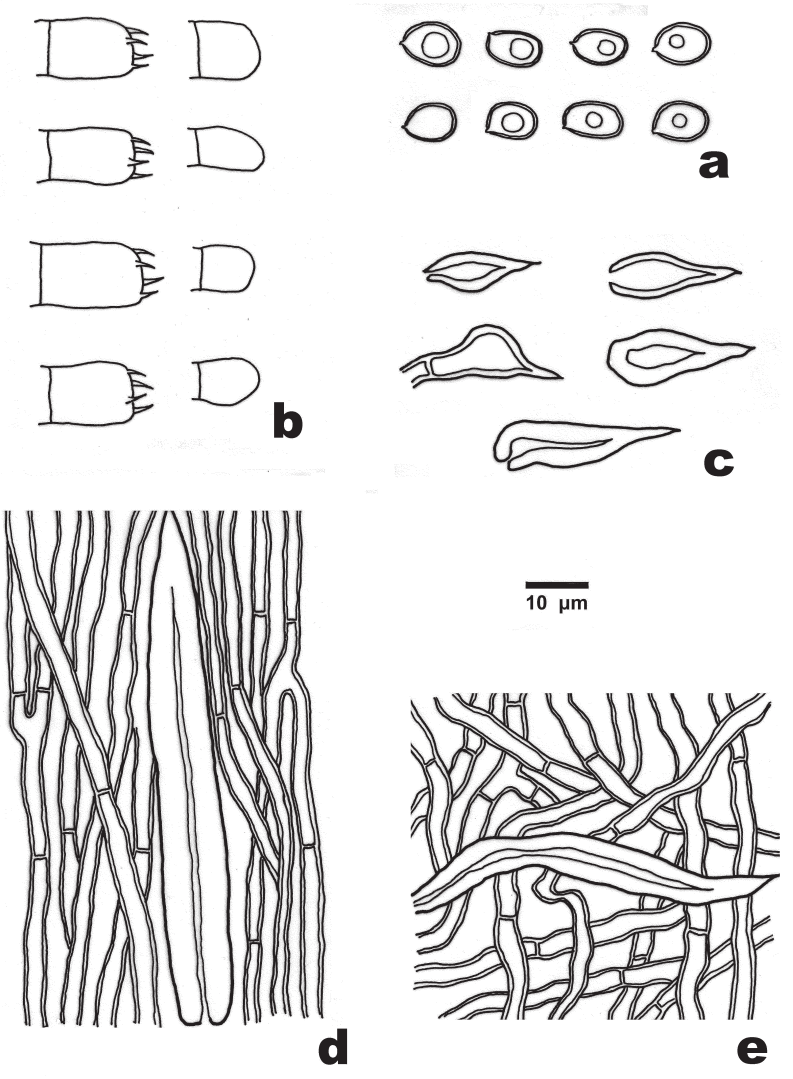

Figure 3. *Inonotus griseus* (holotype).
a: Basidiospores. b: Basidia and basidioles. c: Hymenial setae.
d: Hyphae and hyphoid setae from trama. e: Hyphae and hyphoid setae from subiculum.

Subiculum. Generative hyphae yellowish, slightly thick- to thick-walled with a wide lumen, rarely branched, straight to flexuous, interwoven, strongly

agglutinated, CB+, 3–5 μm in diam. Hyphoid setae present, acute, dark brown, thick-walled, slightly flexuous, up to hundreds μm long, 4–10 μm wide.

Tubes. Generative hyphae yellowish, slightly thick-walled with a wide lumen, occasionally branched, straight, subparallel along the tubes, CB+, 2.5–4 μm in diam. Hymenial setae subulate to ventricose, acute, straight, dark brown, thick-walled with a wide to narrow lumen, 12–32 × 5.5–11 μm; hyphoid setae embedded in hymenium, never penetrating beyond hymenial surface, acute, dark brown, thick-walled, parallel along the tubes, up to hundreds μm long, 12–22 μm wide; cystidia and cystidioles absent; basidia barrel-shaped, with a simple septum and four sterigmata, 13–17 × 8–12 μm; basidioles barrel-shaped to subglobose, slightly smaller than basidia.

Basidiospores ellipsoid, hyaline, slightly thick-walled, smooth, mostly bearing a medium to large guttule, IKI–, CB+, (8.5–)9–10.5(–11) × (6–)6.3–7.2 μm, L = 9.7 μm, W = 6.74 μm, Q = 1.44 (n = 30/1).

Remarks: *Inonotus griseus* has annual and resupinate basidiocarps, a monomitic hyphal system with simple septate generative hyphae, hyphoid and hymenial setae, and ellipsoid, hyaline, slightly thick-walled cyanophilous basidiospores. All these characters fit well with the concept of *Inonotus* as emended by Dai (2010).

Inonotus chihshanyenus T.T. Chang & W.N. Chou, *I. magnisetus* Y.C. Dai, *I. micantissimus* (Rick) Rajchenb., and *I. perchocolatus* Corner share resupinate basidiocarps producing a monomitic hyphal system, and both hyphoid and hymenial setae with *I. griseus*. Compared with *I. griseus, I. chihshanyenus* has larger pores (1–3 per mm), longer hymenial setae (25–60 μm long), and smaller basidiospores (6.5–9 × 5–6.5 μm, Chang & Chou 1998) that were also considered to be diagnostic of *I. chihshanyenus* by Ryvarden (2005); *I. magnisetus,* recently described from Guangdong Province, southern China, has similarly sized pores (5–6 per mm), but its basidiospores are colored and much smaller (4–4.5 × 3.7–4 μm, Dai 2010); *I. micantissimus* also has similarly sized pores (5–7 per mm), but produces colored and larger basidiospores (10–13 × 8–12 μm) and is distributed in tropical America (Ryvarden 2005); *I. perchocolatus* differs mainly in its smaller pores (6–9 per mm) and basidiospores (5–7 × 4.5–5.5 μm, Ryvarden 2005). Our phylogeny (Fig. 1) indicates a close relationship between *I. griseus* and *I. obliquus* (Ach. ex Pers.) Pilát, although with low statistical support. *Inonotus obliquus* differs morphologically from *I. griseus* by its dark reddish-brown pore surface, smaller pores (6–8 per mm), and narrower, hyaline to brownish basidiospores (5.5–6.5 μm, Ryvarden 2005). Therefore, the combination of phylogenetic and morphological evidence supports *Inonotus griseus* as an independent *Inonotus* species.

Inonotus has been traditionally distinguished by an annual growth habit and a monomitic hyphal system from *Phellinus* Quél., another large genus in *Hymenochaetaceae* (Ryvarden 2005). Based on a phylogeny inferred from the nLSU region, Wagner & Fischer (2002) transferred several *Phellinus* species with perennial basidiocarps and a dimitic hyphal system (at least in the trama) into *Inonotus*: *I. baumii* (Pilát) T. Wagner & M. Fisch., *I. linteus* (Berk. & M.A. Curtis) Teixeira, *I. vaninii* (Ljub.) T. Wagner & M. Fisch., and *I. weirianus* (Bres.) T. Wagner & M. Fisch. This classification was followed in several later publications (Dai 2010, Wu et al. 2012, Zhou & Qin 2012, Tian et al. 2013, Vlasák et al. 2013), placing newly described and combining similar species in *Inonotus*. However, based on their ITS and mitochondrial SSU sequence analyses, Jeong et al. (2005) did not accept the above mentioned species with perennial basidiocarps and dimitism in *Inonotus,* considering them to have a sister relationship with *Inonotus*. The generic circumscription of *Inonotus* is still under debate. In our phylogeny (Fig. 1), species (including *I. griseus*) with a monomitic hyphal system in both context/subiculum and trama form the core *Inonotus* clade, while the other sampled *Inonotus* species that are dimitic (at least in trama) form the residual clades A and B. More sampling and additional loci are needed to determine a more exact circumscription of *Inonotus*.

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